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XXXII.—*Further Observations on the Distinctive Characters and Reproductive Phenomena of the Amœban Rhizopods.* By G. C. Wallich, M.D., F.L.S., &c.

Although certain changes have taken place in the aspect of the specimens of *Amœba villosa* still met with in the remaining portion of the Hampstead material in which they were originally discovered, these neither involve the loss of any essential characters, nor can they justly be regarded as anything more than mere changes in degree. As now seen, the individuals have become somewhat reduced in size; they contain less fresh-looking organic food-particles; their movements are more sluggish; the nucleated corpuscles and sarcoblasts are not so frequently met with; the crystalloids, although not less numerous, are smaller; the nucleus is shrunk somewhat, but still retains its very marked membranous capsule; the contractile vesicle performs its diastole and systole at longer intervals; and, lastly, the villi are not so densely studded over the surface of the villous region. But inasmuch as these evidences of impaired vitality are equally manifest in very recently procured specimens, it is probable that they are due to the operation of identical causes—namely, failure of proper nutriment incidental on the season, or temporary stagnation, without absolute drying-up, of the water in which they live.

In the August Number of the 'Annals' (p. 124) I expressed my doubts as to the "reproductive cells" of Mr. Carter (assuming these to be the same bodies to which I had given the name of sarcoblasts) being the product of repeated binary division of the nucleus, and I gave reasons for inclining to an opposite conclusion. My late observations show that these doubts were not unfounded; for whilst I am able to confirm Mr. Carter's statement regarding the recurrence of nuclear division up to a certain limit, I still experience the same difficulty in reconciling

the presence, sometimes of a single, sometimes of multiple encapsuled nuclei, which I have frequently witnessed, with the occurrence in the same individual of a large number of sarcoblasts of nearly uniform average size, but which size differs very materially from the also uniform size of these multiple nuclei. In short, I believe that whilst duplicative division does take place in the nucleus and the nuclear capsule up to a certain point, it stops there—and that the sarcoblasts are formed within the body of the nucleus, and are not segments of it and its capsule combined. This view is strengthened, moreover, by some curious facts which have been observed by me only within the past few days—facts which would seem to indicate that the office of the true nuclei is not identical with that of the sarcoblasts, although in both cases a new brood is the result. How the sarcoblasts escape though the apparently imperforate capsule of the nucleus I am ignorant; but I would remark that in *Acanthometra* and *Thalassicolla* they occur within the nuclear body, as well as externally to its capsule (that is to say, throughout the endosarc generally), whereas amongst a multitude of the last-named organisms examined by me I have not met with a divided or a dividing nucleus in a single instance; and in the Foraminifera and Polycystina, which possess no true nucleus at all, the sarcoblasts ("yellow cells" of authors) are present in great number. It is possible, however, that I may be in error in regarding my sarcoblasts as identical with the "reproductive cells" of Mr. Carter, more particularly as he describes the latter as invariably exhibiting a distinct capsule in their mature state, whereas I have entirely failed to detect any endogenously formed bodies, besides the nucleus or few multiple nuclei, which possess a definite capsular covering. It only remains for me to point out that the sarcoblasts in their early stage present a pale-yellow tint, and are somewhat oily-looking, which is not the case with the multiple nuclear bodies. Additional evidence, however, must be produced before these questions can be regarded as definitively answered.

Recent examples also enable me to corroborate the statement advanced in my previous papers respecting the occasional occurrence of a simultaneous tripartite division of the nucleus. When this happens, it is brought about by the inversion of two folds of the nuclear capsule nearly at right angles to each other, one fold passing completely across so as to isolate a segment, whilst the other bisects the remaining portion. In this manner the three divisions, although originally differing in shape, may each contain equal quantities of nuclear matter, and by the gradual rounding of their outline ultimately assume isometrical proportions. In these instances, and indeed in the case of the multiple

nuclei generally, I have detected no nucleolus, and the granular mass of the nucleus has invariably filled up its own capsule. In no example, whether amongst the youngest or most mature specimens, have I observed the nucleus (that is, the entire nuclear cell and contents) attached to the ectosarc, or the granular mass of the nucleus itself attached at one point to the interior of the capsule containing it,—the granular mass being free, and its component granules merely sustained within a matrix of viscid protoplasm, although frequently these granules cling, as it were, to the interior of the cell, just as the chlorophyll-granules of the vegetable cell, generally speaking, form a layer immediately within the denser granular protoplasm which lines and circulates within its interior.

It will be seen, on reference to Mr. Carter's last paper in the 'Annals' (October 1863, p. 254), that he now entirely relinquishes the character derived from the supposed anomaly in the configuration of the nucleus, on which he so strongly insisted in his reconstruction of the typical characters of *Amœba princeps*, and accepts, as the distinctive feature of that form, the villous appendage and, as a matter of course, the novel phenomena involved in the discovery of its presence in *Amœba villosa*. Further comment upon this is accordingly unnecessary.

Another point on which my previous views have been materially strengthened by recent experience is the nature of the circulation in *Amœba*. I am more than ever convinced that this is not a vital act, but a secondary and mere mechanical effect consequent on the inherent vital contractility of the sarcode. It is only necessary to watch a specimen of *Amœba* carefully, to become convinced that the appearance of a returning as well as an advancing stream of granules is illusory. The stream, it will be observed, is invariably in the direction of the preponderating pseudopodial projections. The particles simply flow along with the advancing rush of protoplasm. There is no return stream; but the semblance of one is engendered by one layer of particles remaining at rest whilst another is moving past them. In short, the effect is similar to that which would be produced were an empty and transparent bladder or caoutchouc sac, containing granular bodies of greater specific gravity than the viscid fluid within which they were sustained, to be rolled along a plane surface. In such a case it is obvious that only those granules on the upper or free aspect of the sac would be carried onwards —that, having arrived at the most advanced point, they would be deposited, as it were, and remain stationary, as would also that portion of the sac on which they rested, until the rest of the mass should have flowed over them again, causing them now to appear at the posterior extremity, when they would once more

be urged onwards as before. The same explanation will, I think, be found to hold good even in the attenuated pseudopodia of some families, as, for instance, the Foraminifera. The essential attributes of sarcode (namely, extensibility and contractility), coupled with the polymorphism evident in every example in which definite form is not partially maintained by the presence of a shell or test, necessarily involve the power of retracting as well as projecting these processes, whereas the tenacity of the substance is not such that a pseudopodium once projected can be retracted towards the body in the same way that a piece of rope thrown forwards from a given point can be hauled in again inch by inch. In the broad pseudopodium of *Amœba*, as also in the attenuated filament of the Foraminifera, or the still more subtle filament of *Acanthometra* or *Euglypha*, the process is the same, and is brought about by a reciprocal outward and inward flow of the sarcode-substance; and thus the granular particles are merely the passive exponents of a vital force which exists quite independently of them. Hence, with all deference to so high an authority as Professor Schultze, I would still regard the circulation of granules in the Rhizopods as a pseudo-cyclosis, analogous, I grant, in appearance, but not in origin, to the cyclosis observable in certain vegetable cells, as for example *Tradescantia*.

Whilst recently endeavouring to establish the relation between the phenomena of the circulation seen in *Amœba* and the cell of *Vallisneria*, the following very singular facts revealed themselves. As is well known, within each cell of *Vallisneria* is to be found—in addition to the more watery portion (or, as Mr. Carter has appropriately termed it, the "axial fluid"), the layer of very finely granular protoplasm which seems to hug and flow round the interior of the wall, and the chlorophyll-granules—a single colourless mass of protoplasm (of considerable size) which, generally speaking, is only partially affected by the cyclosis, but nevertheless sometimes flows round with the chlorophyll-granules and occasionally adheres to the peripheral protoplasm so as to form a nodule on its internal aspect. This mass of protoplasm, which has been termed the nucleus or cytoblast, presents at its centre a nucleolus which may be rendered very palpable by the ordinary chemical reagents, but especially so by solution of magenta. Occasionally also some of the chlorophyll-granules form an investing layer over the surface of the nucleus, remaining adherent to it in such a manner as to prove that their presence is not accidental. This association of chlorophyll-granules and nucleus is very constant in the mature leaf, but, together with the cyclosis and other phenomena now about to be mentioned, is most distinctly visible in the perianth. On examining a delicate lon-

gitudinal section taken from the free margin of the perianth, the gradational changes which take place in the cytoblast, from the free state first described to that in which it is embraced by the green chlorophyll-granules, and, after divesting itself of these bodies, becomes differentiated into a distinct anterior and posterior portion, the former throwing out the flagelliform proboscis of *Astasia*, whereas a regularly pulsating contractile vesicle invariably occupies a position in the latter, are most strikingly manifested. Of the purely endogenous origin of the *Astasia*-like bodies I feel perfectly satisfied, having, as already stated, not only detected the single cytoblast in *every* cell both of the leaf, the spiral flower-stem, and the perianth, but also assured myself by the most careful examination that no lesion had taken place in the cell-walls whereby the entrance of zoospores might have been effected. But the most wonderful feature remains yet to be noticed—namely, the multiplication of the *Astasia*-like bodies by longitudinal fission whilst still within the parent cell, until sometimes as many as ten have been congregated together. In several cases the entire process, which did not occupy more than half an hour, took place under my eyes—commencing at the ciliated extremity, and proceeding backwards until it reached the contractile vesicle, which, after sundry partitions and reunions, finally divided into two halves, one of which was apportioned to each of the new individuals.

Without entering at present into the subsequent history of these bodies, I am desirous of pointing out wherein they resemble and wherein they present marked differences from the characters described by me as pertaining to *Amœba*. Like the Amœban Rhizopods generally, without any exception, whether naked or testaceous, their protoplasmic substance is differentiated into an anterior and posterior portion. Their contractile vesicle discharges itself invariably in the latter region, but, unlike that of the *Amœbæ*, it is fixed in that position permanently. When supplementary contractile vesicles are given off from or near the primary one, they either coalesce with it or discharge themselves independently as in *Amœba*. The nucleus which is in the centre of the body never alters its position. And lastly, whereas the movements in *Amœba* are strictly polymorphous, those of the *Astasia*-like organisms have the power of altering their *outline* only, by extending and contracting the body round an imaginary axis; so that although it is conceivable that the reparation of lost parts may take place, such reparation would consist in the renewal of a determinate and not an indeterminate figure. Coupling this, then, with the absence of any digestive apparatus whatever, we are furnished with a clear line of demarcation between the animal and vegetable, whilst in the simpler forms

of Infusoria the presence of a fixed and determinate aperture or apertures connected with a digestive system sufficiently separates them from the Amœban or highest type of Rhizopod structure.

But, to revert once more to the *Amœbæ*. Within the past fortnight my friend Mr. J. N. Tomkins, the able Inspector of the Government Vaccine Department, called my attention to his having unexpectedly detected a profusion of *Amœbæ*, possessing all the characters of *A. villosa*, in some damp confervoid material which had been scraped off a stone slab in his garden and consigned to a vessel containing water, about two months previously. I confess that, even putting out of the question the untenable theory of "spontaneous generation," the development of these *Amœbæ* from germs either constantly present in damp soil or deposited on it through the medium of wind or rain from distant localities was regarded by me with doubt, if not actual incredulity. For knowing how zealously my friend collects the various microscopic forms of life, it seemed far from improbable that these *Amœbæ* were derived from the refuse cast aside from his aquaria. The sequel, however, showed that my doubts were altogether groundless. But, leaving this point for the present, I may state that the question I was especially desirous of determining—namely, the possibility of a gelatinous organism like an *Amœba* being able to withstand the desiccation to which it must be subject during summer if it be the normal inhabitant of confervoid growth met with in similar positions—was deemed by me sufficiently important to merit immediate inquiry.

On examination of a portion of the material which had been kindly placed at my command, I found it contained an abundant stock of *Amœbæ*, both old and young, and that these exhibited (at a bird's-eye view, as it were) the collective characters of *Amœba radiosa, diffluens, globularis, Schultzii, limax, princeps, guttula, verrucosa, quadrilineata, actinophora,* and *villosa*. But whilst it would have been easy to select individual specimens presenting in a marked degree those purely external characters which have been held to distinguish all but the last-named of these forms, it would have been equally easy to demonstrate, by means of the infinite intermediate varieties, that all are the offspring of a common parent, and that the mere outward deviations in figure and degree of differentiation are dependent on those ever-varying physical conditions to which they are amenable, and which will probably for ever elude our scrutiny.

It is necessary to state explicitly that I lay no claim to the discovery that many Infusoria and some even more highly organized forms (as, for example, Rotifera) undergo desiccation without perishing. Professor Ehrenberg, Dr. B. Hicks, and more recently M. Balbiani in conjunction with Mr. Samuelson,

have clearly established this fact. My object in dwelling on the observations now recorded is to show how inseparably most of the minor distinctions are connected with accidental changes of physical conditions, and how guarded we should be in assigning limits to variation before we have become acquainted with the extent to which such changes may operate.

In my previous papers it was stated that many incidental facts led me to believe in the narrow limitation of species in *Amœba*, if not in their absolute unity. The appearances presented by the specimens now under notice have served to confirm that belief, and I can hardly imagine it possible that any person, viewing them unbiasedly and witnessing the occurrence of extreme variability even in the earliest stage of the *Amœbæ*, when (as seen by me within the past few days) countless numbers of these minute organisms alternately assume the characters of the most "lobose" type and of *Actinophrys*, could arrive at any other conclusion.

But the history of these *Amœbæ* is not left in doubt; for not only did a fresh supply of confervoid material, scraped off and delivered to me as procured, present specimens after being immersed in water for a few days, but (in order to exclude those sources of error that might be supposed to attach to my observations had the supply been obtained from my own garden, where the refuse of aquaria is at times flung out) a portion of confervoid growth taken from another locality*, having been consigned to water for a few days, was found to furnish similar results. Lastly, with a view to put the matter to a still more severe test, a small quantity of the material first obtained from Mr. Tomkins was placed on a plate of glass and left to dry completely by evaporation. In three days it was again covered with water. Twenty-four hours afterwards no traces of life beyond a few young *Chilodontes* and monads were visible. In forty-eight hours *Amœbæ* were observed, although in much smaller number and more sluggish in their movements than was the case prior to this second desiccation; whilst after the lapse of four days the *Amœbæ*, although still less numerous, were as active as ever.

In the confervoid material recently obtained I detected numerous effete *cysts* of *Amœba*, some quite empty and crumpled up into angular folds, some enclosing the empty frustules of a diatom that occurs abundantly in a living condition in the damp soil associated with the confervoid layer—namely, *Nitzschia Amphioxys*; whilst others enclosed sarcoblasts, and in some examples coarsely granular nuclear bodies which I at once

* The spot selected was a gravel walk, at one part of which rain had lodged occasionally. It had, to my knowledge, been repeatedly raked over during the past three months.

recognized as analogous, if not identical in their origin, with the *naked* nuclear masses described and figured by me in a previous Number of the 'Annals' as occasionally resulting from the extra-capsular subdivision of the granular contents of the primary nuclear cell (Annals, May 1863, p. 368).

The presence of these dead and empty frustules of *Nitzschia* is well worthy of note, as yielding evidence, almost tantamount to proof, that their soft contents had undergone digestion, and hence that they had been received into the interior of the *Amœbæ* prior to encystation and desiccation; whilst the fact of their being commonly taken as food by the *Amœbæ* is manifest from the numbers of *Amœbæ* now living on the material, that contain within their endosarc frustules full, as also partially relieved, of their endochrome.

It may be recollected that, in the same Number (*loc. cit.*), I expressed doubts as to the normal investiture of any *Amœba* by a membranous ectosarc, and inclined to the view that the single example of such investiture which had fallen under my notice up to that period betokened encystation. The strongest confirmation of that opinion is hence afforded; for not only were empty and effete cysts now met with, but a few examples of cysts in which the sarcode-mass, although apparently in a deteriorated state, had recovered a sufficient degree of contractility to produce distinct changes in their form. Of the ultimate fate of these revivified cysts I have not as yet been able to satisfy myself. There are reasons, however, for supposing that they are only destined to afford protection to the reproductive elements until such period as the latter are in a fit condition to be set at liberty. It would also seem probable that the encystation of *Amœba* does not take place, as a matter of course, at certain seasons, but only when the normal conditions of the creature's existence become impaired or altogether deficient—the increased consolidation of the external layer being the exceptional result of prolonged contact with the vitiated medium around, and the cessation of that reciprocal interchange between endosarc and ectosarc, described by me under the name of Amœbasis, which is continually going on in the healthy state of the organisms. That such is the case may be reasonably inferred from the absence of anything like the encysted condition in the specimens of *Amœba villosa* which have now been seven months under close observation, and carefully protected from desiccation. Should encystation in the remaining Hampstead material follow on slow desiccation being permitted to take place, the proof required will be complete.

Want of time and space preclude me from entering into full details respecting the several modes in which, in addition to

those previously described under the heads of "gemmation" and "viviparous parturition," a new brood of *Amœbæ* appears to be ushered into existence. For the present, therefore, I would merely state that one series of young individuals seems to be derived from the conversion of each free sarcoblast into a polymorphous body, devoid of cilia or flagelliform organ, but provided from the first with a nucleus, a contractile vesicle, and a rudimentary villous organ—that a second series, less frequent than the last, seems to result from the similar conversion of each encapsuled nucleus into a polymorphous body, in which, besides the organs just enumerated, may frequently be seen two or three spherical masses undistinguishable from the sarcoblasts; whilst a third and by far the most numerous brood appears to be derived from each separate granule of the naked mulberry-like nuclear masses which were described by me in the 'Annals' for May (p. 368) as being occasionally formed within the parent *Amœba*. The evidence of this last-mentioned mode of increase consists in the admixture of minute *Amœbæ* with some of the granules which are still quiescent, in their barely exceeding the latter in size, and especially in the gradual transition observable from the quiescent to the motile and polymorphous condition of the granules. Of course it would be futile to attempt an explanation of these processes in the present state of our knowledge; for, although satisfied of the occurrence of these varied methods of increase, I consider the questions they involve as too important to be solved without much additional information or on mere inferential reasoning.

Lastly, I would mention another interesting fact which has revealed itself within the past few days, but the details of which must be supplied hereafter. I allude to the transition observed to have taken place, in specimens of free *Amœbæ* preserved alive in shallow glass cells, from the naked to the testaceous condition—a form closely resembling, if not identical with, *A. radiosa* having first assumed a state of comparative rest, as if about to become encysted, and then gradually secreted the delicate hyaline outer wall which ultimately presented the unmistakable characters of the test of *Arcella vulgaris*.

Kensington, October 20, 1863.

Note.—I am permitted by Mr. Tomkins to state that, whilst looking over the same material, he has distinctly seen the animal of *Arcella vulgaris* evacuate its test, and move away in the garb of a naked Amœban. This occurrence has often been regarded as probable, but I am not aware that it had previously been actually witnessed by any trustworthy observer.